Designation: E2870 – 19

Standard Practice for Evaluating Relative Effectiveness of Antimicrobial Handwashing Formulations using the Palmar Surface and Mechanical Hand Sampling[1]

This standard is issued under the fixed designation E2870; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This practice covers and is designed to determine the relative effectiveness of antimicrobial handwashing agents in reducing transient microorganisms using a controlled handwash.

1.2 Knowledge of microbiological techniques is required for these procedures.

1.3 This practice is used to evaluate topical antimicrobial handwashing formulations.

1.4 Performance of this procedure requires the knowledge of regulations pertaining to the protection of human subjects (21 CFR Parts 50 and 56).

1.5 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.6 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.* For more specific precautionary statements, see 8.1.

1.7 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

E1054 Test Methods for Evaluation of Inactivators of Antimicrobial Agents

E1174 Test Method for Evaluation of the Effectiveness of Health Care Personnel Handwash Formulations

E2755 Test Method for Determining the Bacteria-Eliminating Effectiveness of Healthcare Personnel Hand Rub Formulations Using Hands of Adults

E2756 Terminology Relating to Antimicrobial and Antiviral Agents

E2784 Test Method for Evaluation of the Effectiveness of Handwash Formulations Using the Paper Towel (Palmar) Method of Hand Contamination

2.2 *Other Standards:*

AATCC Test Method 147 Antibacterial Assessment of Textile Materials: Parallel Streak Method[3]

21 CFR Part 50 Protection of Human Subjects[4]

21 CFR Part 56 Institutional Review Boards[4]

3. Terminology

3.1 *Definitions*—For definitions of terms used in this document, see Terminology E2756.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *active ingredient, n*—a substance added to a formulation specifically for the inhibition or inactivation of microorganisms.

3.2.2 *reference formulation, n*—formulation against which the activity of the test formulation is compared, for example, a handwash without an active ingredient or a handwash with a different active ingredient than the test formulation. This formulation is not considered a standard.

3.2.3 *test material, n*—a product or formulation which incorporates antimicrobial ingredients(s).

3.2.4 *test organism, n*—an applied inoculum of an organism that has characteristics which allow it to be readily identified.

[1] This practice is under the jurisdiction of ASTM Committee E35 on Pesticides, Antimicrobials, and Alternative Control Agents and is the direct responsibility of Subcommittee E35.15 on Antimicrobial Agents.

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

[3] Available from American Association of Textile Chemists and Colorists (AATCC), P.O. Box 12215, Research Triangle Park, NC 27709, http://www.aatcc.org.

[4] Available from U.S. Government Printing Office Superintendent of Documents, 732 N. Capitol St., NW, Mail Stop: SDE, Washington, DC 20401, http://www.access.gpo.gov.

The test organism is used to simulate a transient topical microbial contaminant. It may also be referred to as a marker organism, bacterial simulant, or bacterial contaminant.

4. Summary of Practice

4.1 This practice uses adult subjects who have provided a written informed consent and whose hands have been determined to be free from any apparent damage at the time of participation in the study. Subjects are to refrain from use of any antimicrobials for at least one week prior to the initiation of the test procedure (see 9.3).

4.2 The test compares the activity of the two handwash formulations simultaneously on the same subject under standardized and controlled conditions. Hands of the subjects are artificially contaminated with *Escherichia coli*. One hand of the subject is washed with the reference formulation and the other with the test formulation. The *E. coli* cells remaining on the hands are recovered by the glove juice method of sampling using a mechanical scrubber. In other methods, comparisons between two products are made by testing two equivalent groups of subjects. The objective of this practice is to determine the relative difference between two products tested on the same subjects and not to determine absolute reductions in organism levels. By testing both products at the same time on the same subjects with the same bacterial inoculum, variability is reduced.

4.3 Effectiveness of the test material is determined after a single wash by comparing the numbers of viable test organisms recovered after treatment with it and with the reference formulation. As an example, a cleanser with an active ingredient (test formulation) can be compared to the cleanser without an active ingredient (reference formulation) to determine the effect the active ingredient has on product performance.

4.4 No baseline sampling of the hands is performed in this practice. The inoculum volume to the palms is very small. A volume of 100 µL is applied to each palm and the palms and fingers are rubbed together. Spillage and loss do not occur, and organisms are evenly distributed across the palmar surface after rubbing. As the objective of this practice is to determine the relative difference between products and not absolute reductions, baseline sampling is not performed.

NOTE 1—If an investigator wanted to compare the effect of washing with a product to not washing, this test could be conducted with one hand serving as a baseline sample and the second hand treated with the test product.

4.5 The investigator should be aware that there may be health risks associated with the use of the test organism and precautions similar to those referenced in 8.1 should be undertaken.

5. Significance and Use

5.1 Hand hygiene is important for preventing the spread of many types of infections.

5.2 During routine activities, it is primarily the palmar surface, comprising palms, fingers, and finger pads, of the hands that may become contaminated with transient microorganisms. The contamination could then be transferred to articles touched or handled or to other parts of the body. Palmar contamination is used in Test Method E2784.

5.3 In Test Method E1174, incomplete drying of the experimentally contaminated hands dilutes the applied test product, thus compromising its activity. Application of a smaller volume of the microbial test suspension keeps the soil load to a reasonable level while allowing the hands to become visibly dry prior to application of the test material and reference formulation. These modifications are aimed at producing a better approximation of in-use conditions and a more realistic assessment of the test substance, thus providing a more reliable indication of product performance.

5.4 Unlike Test Methods E1174, E2755, and E2784, this practice enables a direct comparison between two formulations on the same subject. The practice also uses a mechanical scrubbing machine in conjunction with the glove juice technique for more efficient recovery of viable test bacteria from the palms. The mechanical sampling results in greater recovery of bacteria from the palms than conventional recovery methods, such as massaging.

6. Apparatus

6.1 *Colony Counter*—Any of several types may be used, for example, Quebec Colony Counter.

6.2 *Incubator*—Any incubator capable of maintaining the following temperature: 35 ± 2 °C.

6.3 *Shaker Incubator*—Any incubator capable of maintaining the following temperature: 35 ± 2 °C and capable of shaking the culture medium at 120 to 140 r/min.

6.4 *Sterilizer*—Any suitable steam sterilizer capable of producing the conditions of sterilization.

6.5 *Timer (Stop-Clock)*—Type that can be read for minutes and seconds.

6.6 *Handwashing Sink*—A sink of sufficient size to permit subjects to wash without touching hands to sink surface or other subjects.

6.6.1 *Water Faucet(s)*—To be located above the sink at a height which permits the hands to be higher than the elbow during the washing procedure. Faucet should maintain a flow rate of 3 L/min as determined in 10.4.

6.6.2 *Water Temperature Regulator and Temperature Monitor*—To set and maintain the water temperature at 40 ± 2 °C.

6.7 *Vortex Mixer*—Any suitable vortex mixer capable of mixing sample and diluent.

6.8 *Mechanical Scrubber*[5]—To mechanically sample the palms for test bacteria (see Fig. 1). The machine contains two artificial metallic paddles covered with an artificial turf for a

[5] The sole source of supply of the apparatus known to the committee at this time is Trishul Equipment, Shiva Industrial Estate, Unit No. 107, First Floor, Lake Road, Bhandup West, Mumbai-400078, India. If you are aware of alternative suppliers, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,[1] which you may attend.

FIG. 1 Mechanical Scrubber

smooth and nonslip surface. The drive mechanism is powered by a 48V DC geared motor and has a variable speed from 50 to 150 r/min produced by an electronic speed regulator. The specialized eccentric mechanism produces 12.7 cm long (total stroke-length) horizontal reciprocating silent movement. Both paddles face upwards, parallel to the palms, and during the test, the complete system works to simulate the live activity of mechanically sampling the hands.

6.9 *Spectrophotometer*—An instrument that can measure optical density at a wavelength of 620 nm.

6.10 *Adjustable or Fixed Volume Pipets and Sterile Tips*—1 mL capacity and 0.1 mL capacity.

6.11 *Sampling Containers*—Any sterile or sterilizable container having a tight closure and sufficient capacity to hold 75 mL of sampling solution (7.2).

6.12 *Centrifuge*—For the sedimentation of *E. coli* for concentration.

6.13 *Sterile Centrifuge Tubes*—Minimum of 15-mL capacity.

6.14 *Sterile Container*—Any sterile or sterilizable container having the capacity to culture the amount of inoculum required for testing.

6.15 *Gloves*—Loose-fitting, unlined, powder-free gloves which possess no antimicrobial properties, or equivalent. Perform a zone of inhibition test, such as AATCC Test Method 147, to evaluate the antibacterial activity. (Plastic bags (6.16) with low bioburden may be used in place of gloves.)

6.16 *Plastic Bags*—May be used in place of gloves. Bags should be approximately 30 × 18 cm, possess no antimicrobial properties and have a low bioburden. Perform a zone of inhibition test, such as AATCC Test Method 147, to evaluate the antibacterial activity.

6.17 *Wrist Ties or Tourniquets*—Any item which will allow the gloves (6.15) or plastic bags (6.16) to be secured to the subject's wrists.

6.18 *Sterile Tissues or Paper Towels*—Any sterile tissue or paper towel that can be used to dry hands.

7. Reagents and Materials

7.1 *Test Substances*—Follow the manufacturer's directions for use of the test material and reference formulation. If directions are not available, use the directions provided in this practice (10.5).

7.2 *Sampling Solution*—Dissolve 0.4 g monopotassium phosphate (KH_2PO_4), 10.1 g disodium hydrogen phosphate (Na_2HPO_4), 1.0 g isooctylphenoxypolyethoxyethanol (for example, Triton X-100), and appropriately validated neutralizers in distilled water. Adjust pH to 7.8 ± 0.1 with 0.1 *N* hydrochloric acid (HCl) or 0.1 *N* sodium hydroxide (NaOH) and bring volume to 1 L with distilled water. Sterilize in an autoclave and aseptically dispense 40-mL and 35-mL portions into sterile sampling containers (6.11).[6]

NOTE 2—A neutralizer validation should be conducted according to Test Method E1054 prior to the study. Test Method E1054 provides a list of neutralizers appropriate for commonly used antimicrobial agents. In some cases, neutralization may be achieved by dilution alone.

7.3 *Dilution Fluid*—Sterile Butterfield's buffered phosphate diluent[7] (or other suitable diluent) adjusted to pH 7.2 ± 0.1 and containing an effective inactivator for the test material, if necessary.

NOTE 3—Inactivator is only required if neutralization of the test material cannot be achieved upon dilution into the sampling solution (see 7.2).

7.4 *Soybean-Casein Digest Agar with MUG* (4–methylumbelliferyl-b-D-glucuronide)—Sterile tryptic soy agar with MUG (0.5 g/L), used for the indication, recovery and growth of *Escherichia* species or other solid media appropriately validated to support the growth of the test organism. With appropriate neutralizers, if required, according to Test Method E1054.

NOTE 4—The MUG substrate is hydrolyzed by b-D-glucuronidase to yield a fluorescent end product, 4-methylumbelliferone. b-D-glucuronidase is possessed by *E. coli* (ATCC 10536).

7.5 *Broth*—Sterile soybean-casein digest broth (tryptic soy broth) or other liquid media appropriate to support growth of the test organism.

7.6 *Soybean-Casein Digest Agar*—Sterile tryptic soy agar for growth of *Escherichia* species or other solid media appropriately validated to support the growth of the test organism.

7.7 *Physiological Saline*—Sterile. Used to prepare the final inoculum.

7.8 *Ethanol or Isopropyl Alcohol Solution*—70 % ethanol or isopropyl alcohol in water (v/v) for hand decontamination.

7.9 *Chlorhexidine Skin Cleanser*—Antiseptic skin cleanser containing 4 % chlorhexidine gluconate (w/v) for hand decontamination.

8. Test Organism

8.1 *Escherichia coli* (ATCC 10536) is the test organism. The plating agar should be soybean casein digest agar with MUG (7.4) or another suitable indicator. (**Warning**—The application of microorganisms to the skin may involve a health risk. Prior to applying the test organism to the skin, the antibiotic sensitivity profile of the strain should be determined.

[6] Peterson, A. F., "The Microbiology of the Hands: Evaluating the Effects of Surgical Scrubs," *Developments in Industrial Microbiology*, Vol 14, 1973, pp. 125–130.

[7] Butterfield, C. T., "The Selection of a Dilution Water for Bacteriological Examinations," *J. Bacteriol*, Vol 23, 1931, pp. 355–368.